

LISTING OF THE CLAIMS

Please replace this listing of the claims in lieu of all previous listings.

1. (Currently amended) An immunogenic composition comprising a live *Brucella* host cell having a rough phenotype, which host cell contains at least two mutations so as to effect sufficient attenuation ~~is sufficiently attenuated such~~ that upon exposure to a mammal the host cell will not exhibit full virulence of non-attenuated *Brucella*, wherein the host cell is transformed with a recombinant DNA construct replicable in *Brucella*, which DNA construct comprises:

- (i) a promoter recognizable by *Brucella*, and
- (ii) a complementation DNA fragment which encodes a peptide required for lipopolysaccharide O-sidechain synthesis so as to effect lipopolysaccharide O-sidechain synthesis in vivo and which is operably linked to the promoter and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in the host cell,

wherein the association between the *Brucella* host cell and the DNA construct is such that following exposure to a mammal the DNA construct gradually separates from the *Brucella* host cell, whereupon the *Brucella* host cell reverts to a rough phenotype that is rapidly and safely cleared from the mammal.

2. The immunogenic composition of claim 1, wherein the *Brucella* host cell comprises a *Brucella* DNA fragment containing a stable non-reverting deletion mutation, having the nucleotide sequence of SEQ ID NO: 1 modified to delete nucleotides from position 1067 to position 1671.

3. The immunogenic composition of claim 1, wherein the *Brucella* host cell is *Brucella melitensis*.

4. The immunogenic composition of claim 1, wherein the *Brucella* host cell is WRRP1, having ATCC accession number PTA-3753.
5. The immunogenic composition of claim 4, wherein *Brucella* host cell WRRP1 has no antibiotic resistance markers.
6. (Canceled) The immunogenic composition of claim 1, wherein the *Brucella* host cell is WRR51, having ATCC accession number PTA-3754.
7. (Canceled) The immunogenic composition of claim 6, wherein *Brucella* host cell WRR51 has no antibiotic resistance markers.
8. The immunogenic composition of claim 1, wherein the promoter is a *Brucella* promoter.
9. The immunogenic composition of claim 1, wherein the complementation DNA fragment comprises the *wboA* gene.
10. (Canceled) The immunogenic composition of claim 9, wherein the *wboA* complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis.
11. (Currently amended) An immunogenic composition comprising a live attenuated *Brucella* host cell having a rough phenotype, which host cell contains at least two mutations so as to effect sufficient attenuation ~~is sufficiently attenuated such~~ that upon exposure to a mammal the host cell will not exhibit full virulence of non-attenuated

*Brucella*, wherein the host cell is transformed with a recombinant DNA construct replicable in *Brucella*, which DNA construct comprises:

- (i) a DNA fragment operably linked to a first promoter recognizable by *Brucella*, and encoding a heterologous antigen; and
- (ii) a complementation DNA fragment which encodes a peptide required for lipopolysaccharide O-sidechain synthesis so as to effect lipopolysaccharide O-sidechain synthesis in vivo and which is operably linked to a second promoter recognizable by *Brucella*, and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in the host cell cell,

wherein the association between the *Brucella* host cell and the DNA construct is such that following exposure to a mammal the DNA construct gradually separates from the *Brucella* host cell, whereupon the *Brucella* host cell reverts to a rough phenotype that is rapidly and safely cleared from the mammal.

12. The immunogenic composition of claim 11, wherein the *Brucella* host cell comprises a *Brucella* DNA fragment containing a stable non-reverting deletion mutation, having the nucleotide sequence of SEQ ID NO: 1 modified to delete nucleotides from position 1067 to position 1671.

13. The immunogenic composition of claim 11, wherein the *Brucella* host cell is *Brucella melitensis*.

14. The immunogenic composition of claim 11, wherein the *Brucella* host cell is WRRP1, having ATCC accession number PTA-3753.

15. The immunogenic composition of claim 11, wherein *Brucella* host cell WRRP1 has no antibiotic resistance markers.

16. (Canceled) The immunogenic composition of claim 11, wherein the *Brucella* host cell is WRR51, having ATCC accession number PTA-3754.

17. (Canceled) The immunogenic composition of claim 16, wherein *Brucella* host cell WRR51 has no antibiotic resistance markers.

18. The immunogenic composition of claim 11, wherein the promoter is a *Brucella* promoter.

19. (Currently amended) The immunogenic composition of claim 11, wherein the heterologous antigen is selected from the group consisting of anthrax antigens, *Yersinia pestis* F1 and V antigens and F1-V fusion proteins, malaria circumsporozoite and merozoite antigens, *Plasmodium berghei* antigens, *Plasmodium falciparum* antigens, *Plasmodium vivax* antigens, *Plasmodium malariae* antigens, *Francisella* antigens, staphylococcal and streptococcal enterotoxin fragment antigens; *Burkholderia* antigens, *Coxiella* antigens, *Clostridium* epsilon toxoids, botulinum toxoids, smallpox antigens, mycobacterial antigens, cancer antigens, HIV antigens, tetanus toxoids, diphtheria toxoids, pertussis toxoid, *Helicobacter* antigens, *Borrelia* antigens, *Legionella* antigens, *Bartonella* antigens, vaccinia antigens, antigen-GFP fusions, tagged antigens 6his and V5, and fusions of antigens to secretory signals, ~~and genes encoding therapeutic molecules or enzymes producing therapeutic molecules.~~

20. The immunogenic composition of claim 19, wherein the anthrax antigen is selected from the group consisting of *Bacillus anthracis* protective antigen and inactive variants of Edema Factor and Lethal Factor.

21. The immunogenic composition of claim 19, wherein the malaria antigens are CSP and MSP1 antigens of *Plasmodium berghei*, *Plasmodium falsiparum*, *Plasmodium vivax*, or *Plasmodium malariae*.

22. (Canceled) The immunogenic composition of claim 19, wherein the DNA fragment of (i) encodes an enzyme synthesizes lipids and/or polysaccharides.

23. The immunogenic composition of claim 11, wherein the complementation DNA fragment comprises the *wboA* gene.

24. (Canceled) The immunogenic composition of claim 23, wherein the *wboA* complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis.

25. (Currently amended) A vaccine against infection by brucellosis, comprising a live *Brucella* host cell having a rough phenotype, which host cell contains at least two mutations so as to effect sufficient attenuation ~~is sufficiently attenuated such~~ that upon exposure to a mammal the host cell will not exhibit full virulence of non-attenuated *Brucella*, wherein the host cell is transformed with a recombinant DNA construct replicable in *Brucella*, which DNA construct comprises:

- (i) a promoter recognizable by *Brucella*, and
- (ii) a complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis so as to effect lipopolysaccharide O-sidechain synthesis in vivo and which which is operably linked to the promoter and which complements a rough-

conferring mutation in the host cell, thereby effecting a smooth phenotype  
in the host cell,

wherein the association between the *Brucella* host cell and the DNA construct is such that following exposure to a mammal the DNA construct gradually separates from the *Brucella* host cell, whereupon the *Brucella* host cell reverts to a rough phenotype that is rapidly and safely cleared from the mammal.

26. The vaccine of claim 25, wherein the *Brucella* host cell comprises a *Brucella* DNA fragment containing a stable non-reverting deletion mutation, having the nucleotide sequence of SEQ ID NO: 1 modified to delete nucleotides from position 1067 to position 1671.

27. The vaccine of claim 25, wherein the *Brucella* host cell is *Brucella melitensis*.

28. The vaccine of claim 25, wherein the *Brucella* host cell is WRRP1, having ATCC accession number PTA-3753.

29. The vaccine of claim 28, wherein *Brucella* host cell WRRP1 has no antibiotic resistance markers.

30. (Canceled) The vaccine of claim 28, wherein the *Brucella* host cell is WRR51, having ATCC accession number PTA-3754

31. (Canceled) The vaccine of claim 30, wherein *Brucella* host cell WRR51 has no antibiotic resistance markers.

32. The vaccine of claim 25, wherein the promoter is a *Brucella* promoter.

33. The vaccine of claim 25, wherein the complementation DNA fragment comprises the *wboA* gene.

34. (Canceled) The vaccine of claim 33, wherein the *wboA* complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis.

35. (Currently amended) The ~~immunogenic composition~~ vaccine of claim ~~[[34]]~~ 25, wherein when the vaccine is administered to a vaccinee, the lipopolysaccharide O-sidechain polysaccharide is produced in vivo and an antibody to the lipopolysaccharide O-sidechain polysaccharide is produced by the vaccinee in response.

36. (Currently amended) A vaccine against infection by brucellosis and/or a non-brucellosis disease, comprising a live attenuated *Brucella* host cell having a rough phenotype, which host cell contains at least two mutations so as to effect sufficient attenuation ~~is sufficiently attenuated such~~ that upon exposure to a mammal the host cell will not exhibit full virulence of non-attenuated *Brucella*, wherein the host cell is transformed with a recombinant DNA construct replicable in *Brucella*, which DNA construct comprises:

- (i) a DNA fragment operably linked to a first promoter recognizable by *Brucella*, and encoding a heterologous antigen, and
- (ii) a complementation DNA fragment which encodes a peptide required for lipopolysaccharide O-sidechain synthesis so as to effect lipopolysaccharide O-sidechain synthesis in vivo and which is operably linked to a second promoter recognizable by *Brucella*, and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in the host cell,

wherein the association between the *Brucella* host cell and the DNA construct is such that following exposure to a mammal the DNA construct gradually separates from the *Brucella* host cell, whereupon the *Brucella* host cell reverts to a rough phenotype that is rapidly and safely cleared from the mammal.

37. The vaccine of claim 36, wherein the *Brucella* host cell comprises a *Brucella* DNA fragment containing a stable non-reverting deletion mutation, having the nucleotide sequence of SEQ ID NO: 1 modified to delete nucleotides from position 1067 to position 1671.

38. The vaccine of claim 36, wherein the *Brucella* host cell is *Brucella melitensis*.

39. The vaccine of claim 36, wherein the *Brucella* host cell is WRRP1, having ATCC accession number PTA-3753.

40. The vaccine of claim 39, wherein *Brucella* host cell WRRP1 has no antibiotic resistance markers.

41. (Canceled) The vaccine of claim 36, wherein the *Brucella* host cell is WRR51, having ATCC accession number PTA-3754

42. (Canceled) The vaccine of claim 41, wherein *Brucella* host cell WRR51 has no antibiotic resistance markers.

43. The vaccine of claim 36, wherein the promoter is a *Brucella* promoter.



44. (Currently amended) The vaccine of claim 36, wherein the heterologous antigen is selected from the group consisting of anthrax antigens, *Yersinia pestis* F1 and V antigens and F1-V fusion proteins, malaria circumsporozoite and merozoite antigens, *Plasmodium berghei* antigens, *Plasmodium falsiparum* antigens, *Plasmodium vivax* antigens, *Plasmodium malariae* antigens, *Francisella* antigens, staphylococcal and streptococcal enterotoxin fragment antigens; *Burkholderia* antigens, *Coxiella* antigens, *Clostridium* epsilon toxoids, botulinum toxoids, smallpox antigens, mycobacterial antigens, cancer antigens, HIV antigens, tetanus toxoids, diphtheria toxoids, pertussis toxoid, *Helicobacter* antigens, *Borrelia* antigens, *Legionella* antigens, *Bartonella* antigens, vaccinia antigens, antigen-GFP fusions, tagged antigens 6his and V5, and fusions of antigens to secretory signals, ~~and genes encoding therapeutic molecules or enzymes producing therapeutic molecules.~~

45. (Currently amended) The ~~immunogenic composition~~ vaccine of claim 44, wherein the anthrax antigen is selected from the group consisting of *Bacillus anthracis* protective antigen and inactive variants of Edema Factor and Lethal Factor.

46. (Currently amended) The ~~immunogenic composition~~ vaccine of claim 44, wherein the malaria antigens are CSP and MSP1 antigens of *Plasmodium berghei*, *Plasmodium falsiparum*, *Plasmodium vivax*, or *Plasmodium malariae*.

47. The vaccine of claim 36, wherein the complementation DNA fragment comprises the *wboA* gene.

48. (Canceled) The vaccine of claim 47, wherein the *wboA* complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis.

49. (Currently amended) The ~~immunogenic composition~~ vaccine of claim [[48]] 36, wherein when the vaccine is administered to a vaccinee, the lipopolysaccharide O-sidechain polysaccharide is produced in vivo and an antibody to the lipopolysaccharide O-sidechain polysaccharide is produced by the vaccinee in response.

50. (Canceled) A recombinant DNA construct replicable in *Brucella*, which DNA construct comprises:

- (i) a promoter recognizable by *Brucella*, and
- (ii) a complementation DNA fragment which is operably linked to the promoter and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in a host cell transformed therewith.

51. (Canceled) The recombinant DNA construct of claim 50, wherein the complementation DNA fragment comprises the *wboA* gene.

52. (Currently amended) A recombinant DNA construct replicable in *Brucella*, which DNA construct comprises:

- (i) a DNA fragment operably linked to a first promoter recognizable by *Brucella*, and encoding a heterologous antigen, and
- (ii) a complementation DNA fragment which encodes a peptide required for lipopolysaccharide O-sidechain synthesis so as to effect lipopolysaccharide O-sidechain synthesis in vivo and which is operably linked to a second promoter recognizable by *Brucella*, and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in a host cell transformed therewith.

53. The recombinant DNA construct of claim 52, wherein the complementation DNA fragment comprises the *wboA* gene.

54. (Currently amended) The recombinant DNA construct of claim 52, wherein the heterologous antigen is selected from the group consisting of anthrax antigens, *Yersinia pestis* F1 and V antigens and F1-V fusion proteins, malaria circumsporozoite and merozoite antigens, *Plasmodium berghei* antigens, *Plasmodium falsiparum* antigens, *Plasmodium vivax* antigens, *Plasmodium malariae* antigens, *Francisella* antigens, staphylococcal and streptococcal enterotoxin fragment antigens; *Burkholderia* antigens, *Coxiella* antigens, *Clostridium* epsilon toxoids, botulinum toxoids, smallpox antigens, mycobacterial antigens, cancer antigens, HIV antigens, tetanus toxoids, diphtheria toxoids, pertussis toxoid, *Helicobacter* antigens, *Borrelia* antigens, *Legionella* antigens, *Bartonella* antigens, vaccinia antigens, antigen-GFP fusions, tagged antigens 6his and V5, and fusions of antigens to secretory signals, ~~and genes encoding therapeutic molecules or enzymes producing therapeutic molecules.~~

55. (Currently amended) The ~~immunogenic composition~~ recombinant DNA construct of claim 54, wherein the anthrax antigen is selected from the group consisting of *Bacillus anthracis* protective antigen and inactive variants of Edema Factor and Lethal Factor.

56. (Currently amended) The ~~immunogenic composition~~ recombinant DNA construct of claim 54, wherein the malaria antigens are CSP and MSP1 antigens of *Plasmodium berghei*, *Plasmodium falsiparum*, *Plasmodium vivax*, or *Plasmodium malariae*.

57. (Canceled) A host cell transformed with a recombinant DNA construct of claim 50.

58. A host cell transformed with a recombinant DNA construct of claim 52.

Claims 59-68. (canceled)

69. DNA construct pGSG5.

70. (New) The immunogenic composition of claim 1, wherein the DNA construct would be cleared out from a mammal in about eight weeks or less.

71. (New) The immunogenic composition of claim 1, wherein the *Brucella* host cell contains three mutations.

72. (New) The immunogenic composition of claim 11, wherein the DNA construct would be cleared out from a mammal in about eight weeks or less.

73. (New) The immunogenic composition of claim 11, wherein the *Brucella* host cell contains three mutations.

74. (New) The vaccine of claim 25, wherein the DNA construct would be cleared out from a mammal in about eight weeks or less.

75. (New) The vaccine of claim 25, wherein the *Brucella* host cell contains three mutations.

76. (New) The vaccine of claim 36, wherein the DNA construct would be cleared out from a mammal in about eight weeks or less.

77. (New) The vaccine of claim 36, wherein the *Brucella* host cell contains three mutations.